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THE REPERCUSSION OF COLD ATMOSPHERIC PLASMA IN RAT'S SKIN SMOOTHING

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Abstract

The main aim of the present paper is to study the effect of cold atmospheric plasma on rats skin smoothing. The work done by investigation to the triglycerides level in the blood. The samples of blood taken from rats exposed to plasma and a determination to vitamin D as well as calcium concentrations also exists. A calibration samples also used for comparison process. Samples of study prepared and exposed to plasma for certain periods of time. The periods of time of exposure were from (2 - 10 sec) step 2 sec for one week. At the end of exposure to cold plasma, a certain diagnosis for skin sampled done using textural features. The histology of images analyzed by using Energy calculations, moment of inertia, local Homogeneity and correlation. Results show that these are no significant changes during the time periods from 2 – 6 sec/day. The results changed after (8 – 10 sec/day). From vitamin D levels in serum, calcium levels, and Triglycerides in blood serum, the cold plasma make the rats skin smooth and become less dryer with good enhancement. In other words, the cold plasma has a significant effect on skin texture.

Keywords: Cold Plasma, Fats, Skin, Histology, Textural Analysis, Textural Features.

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Introduction

Plasma, the fourth state of matter, from the time of discovered, was in focus point to scientists. They found many processes for plasma generation. Plasma consists of active species that are ions, molecules, radical species, electric field, visible and ultraviolet light. These species make plasma has effects on and in many fields in human life. Researchers make many studies and found wide of applications of cold atmospheric plasma. Such applications are (but not limited) industry, food and medicine. Many researches has been done for these purposes. In industry, plasma used widely in thin films production, depositions and in nano-catalytic manufacturing [1-2]. In environment, cold plasma used in water purification and treatment from contaminations such as bacteria [3]. Also, cold plasma used in ozone generation from water. High-temperature plasmas are used for sterilization of medical devices [4–10]. Cold atmospheric pressure plasmas also be used in medicine by direct apply or indirect. for as for the viable tissues treatment and for biological purification [11-14]. Also, plasma is used in other applications such as improvement of metal oxides in the eggshells [15-16]. Researchers study the effect of plasma on skin moisture and water loss in skin layers [15-17].

Experimental method:

The experimental part includes the following steps:

The six rats used in the study. The rats were kept at the animal house in Al-Nahrian University and used in the lab only at working and exposure to plasma. The rats at the same age and sex (5 months, female albino).A certain part of the rat's skin chosen for the study. The hair shaved to guarantee the skin exposed directly to cold plasma. A control blood sample used with 2ml volume collected and kept in the tube that is for biological tests. Time period used for the exposure part from 2 – 10 sec with step 2 seconds.

Figure (1) shows the experimental rat after hair shaving.

Also in addition to the previous points, a skin samples of the exposed positions have been kept in formalin solution as shown in figure (2) in order to be used for image analysis and to compared with blood results.

Figure (2) shows the skin samples in the formalin solution

Preparation of blood samples: collection of blood samples with volume equal to 2 ml. Centrifuge used with (6000 cycle/mint) for 10 mints to get the serum of blood samples. Laboratory examination done for blood serum to read triglycerides, vitamin D and calcium levels. The examination for blood serum done before and after exposure to cold plasma.

For the plasma exposure part, a plasma source as a plasma jet was used with voltage (175v) for time steps and along week. A 2 cm distance have been approved between the cold plasma source and the mice skin.

For skin image study, skin tissue from animal samples were taken with 2 cm^3 and put in formalin with concentration 10%, then dehydrated by ethanol. After that the samples embedded in paraffin wax, section thickness by a Microtome device with (3-5µm) width and stains by colored dye. The slide container for animal skin tissue after that be ready to obtain histo-image which were taken by using optical light microscope (Olympus/Japan).

Traditional segmentation method used to get texture image for skin samples.

The Moment of inertia, the contrast (A1) is equal to[13][18]:

$$A1 = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} (i-j)^2 \; P(i,j)$$

Where p(i,j): is the normalized image and Ng: The gray level value and equal to 256.

The Logical homogeneity (A2) is calculated using the relation:

$$A2 = \sum_{i=0}^{Ng} \sum_{j=0}^{Ng} \frac{p(i,j)}{1+(i-j)2}$$

The correlation and the energy were calculated using the equations:

$$A3 = \sum_{i=0}^{Ng} \sum_{i=0}^{Ng} \frac{p(i,j)(i-Mx)(j-My)}{\sigma x - \sigma y}$$

$$Energy = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} [p(i,j)2]$$

Results and Discussion

Studying cold plasma effects on skin is the main scope of this work. The skin fats is one of the important factors that give indication to skin health and play role in skin enhancement. Measuring the total cholesterol level in the blood serum give good indication about fats buildup in skin layers. Vitamins that are contributes in skin health. Vitamin D

and Calcium levels in serum blood were measured before exposure to cold plasma and after that. Table (1) shows results of blood serum total cholesterol. Also Figure (3) represents the graph of results. From table and figure, the calcium levels in blood still the same for the first four periods of time i.e (2-8) sec. as the time reached to 10 sec/day, the calcium levels changed and increased. Table (2) shows the vitamin D levels in blood serum for different period of exposure (sec/day). Figure (4) presents the graphical description of D vitamin levels. From table (2) and figure (4), the D vitamin levels also didn't change sufficiently during the periods 2,4,6 sec/day. At period 8 sec/day, the vitamin begins to changed levels and still increased at time 10 sec/day period. This means that, the cold plasma energy that is applied directly on rats skin make a deposition to vitamin D and this cause the calcium levels to be building up gradually. Triglycerides, a type of fats, also recorded in our work. Table (3) presents the results of these fats in sample. Results showed that the levels of Triglycerides was before exposure to cold plasma as (7.2±2.0 a, 6.3 a). after exposure to cold plasma periods, it recorded (7.8±0.4 b, 6.8b). results of Triglycerides record changes in times at that in Vitamin D levels periods of change i.e at 8 – 10 sec/day. The moment of inertia calculated by texture features shows high values (2.40995, 2.3793, 2.4594) for first three groups (2,3,4) respectively for groups treated with cold plasma for periods (2,4,6)sec/day. and recorded lowest values (1.8206, 1.7726) as the period of exposure increased to (8 ,10)sec/day. Thses results gives indication to that the skin surface become more smooth due to the effect of cold plasma. For the correlation, it recorded high value at (0.8213) for group number 6 that exposed to plasma for period (10 second). With comparing with other groups and control which treated 10 sec/day for 1 week, it highest than these. The meaning of this is that fat texture pixels were correlated with other neighboring pixels. In other words, the skin become refined. The texture analysis of images histology showed highest values in group no. 6 for calculations of the energy as well as the local homogeneity. The meaning of this is that all the gray lever pixel has almost the same value. That is the homogeneity of the skin and the intensity difference between the pixels as good as cease to exist.

Table (1) calcium levels in blood serum befor and after cold plasma exposure.

Group number	Exposure period (sec/day)	before mg/dl	After (one week) mg/dl
1 (control)	-	3.9±0.5 a	3.8 ±1.2 a
2	2	3.7 ±1.0A a	3.6±3.0 a
3	4	4.4±1.2 a	4.5±2.1a
4	6	4.5±0.2 a	4.6±1.1 a
5	8	5.1±2.0 a	5.1±2.0 a
6	10	5.3±0.2 a	5.7±2.0 b

Table (2) Vitamin D levels in blood serum before and after cold plasma exposure.

Group number	Period of the exposure(sec/day)	before ng/dl	After (one week) ng/dl
1 (control)	-	6.3±1.0 a	6.2±1.2a
2	2	6.2±1.0 a	6.1±1.1 a
3	4	6.0±1.2 a	6.0 ±1 a
4	6	5.5±0.2a	5.6 ±2.1a
5	8	6.2±0.8 a	6.5±1.0b
6	10	6.4±0.2a	6.7±0.1b

Table (3) values of Triglycerides in blood serum.

Group number	Exposure periods (sec/day)	before exposure mg/dl	After exposure (one week)mg/dl
1 (control)	-	7.3±1.5a	7.2±1.2a
2	2	6.4±1.0 a	6.4±1.0a
3	4	7.9±1.2 a	8.0 ±1a
4	6	6.5±2.0 a	6.4±0.4a
5	8	7.2±2.0 a	7.8±0.4 b
6	10	6.3 a	6.8 b

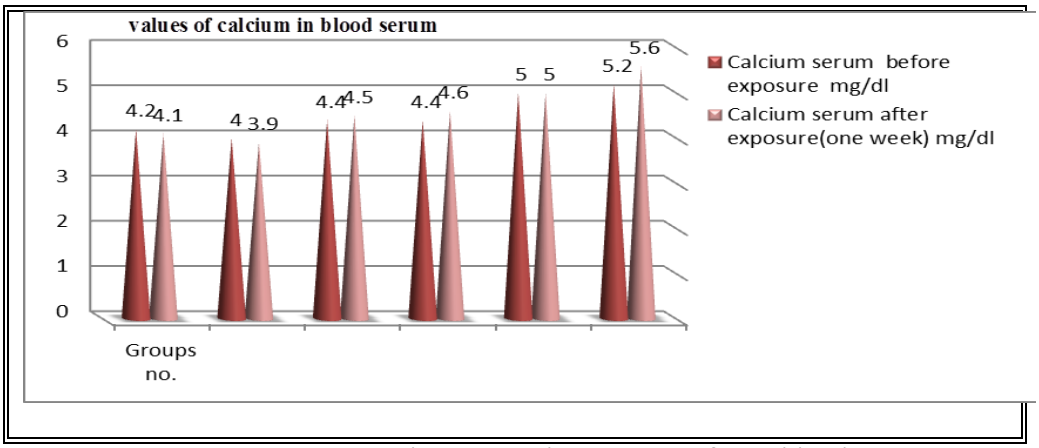

Figure (3) Calcium Levels in serum of rats blood.

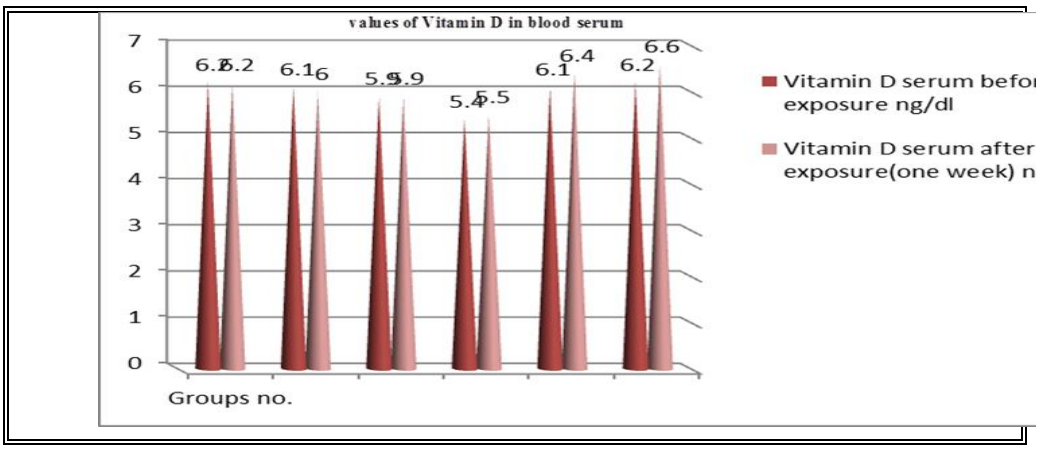

Figure (4) Vitamin D Levels in serum of rats blood.

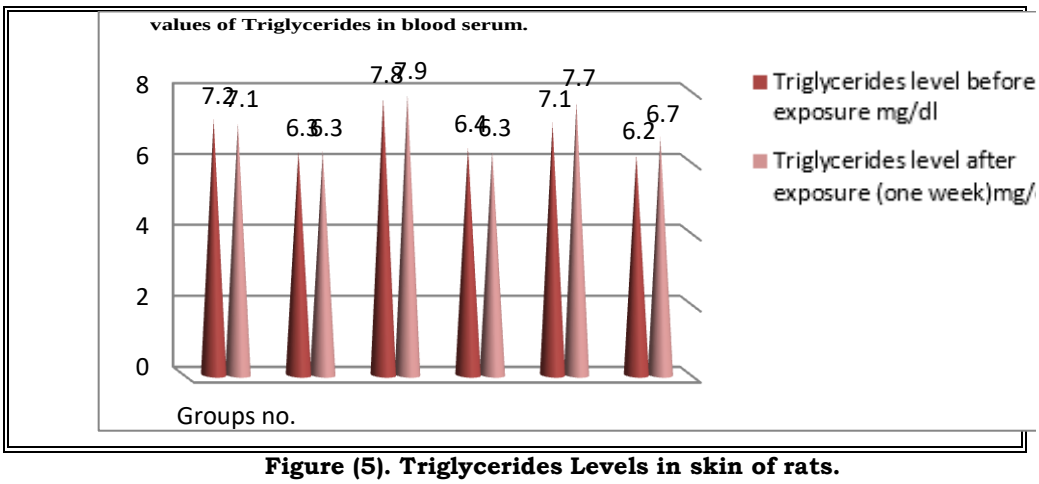

Figure (5). Triglycerides Levels in skin of rats.

Table (4) Texture analysis of histo-images for rats skin.

Groups	exposure period (sec/day)	Threshold values	moment of inertia	Correlation	Energy	local Homogeneity
1	(control)	120	1.4005	0.6572	0.28955	0.90785
2	2	140	2.49885	0.6296	0.2279	0.8446
3	4	130	2.3682	0.47555	0.28505	0.83575
4	6	130	2.4485	0.5261	0.2793	0.8426
5	8	120	2.19306	0.5254	0.25085	0.84785
6	10	130	1.9817	0.8434	0.29635	1.0185

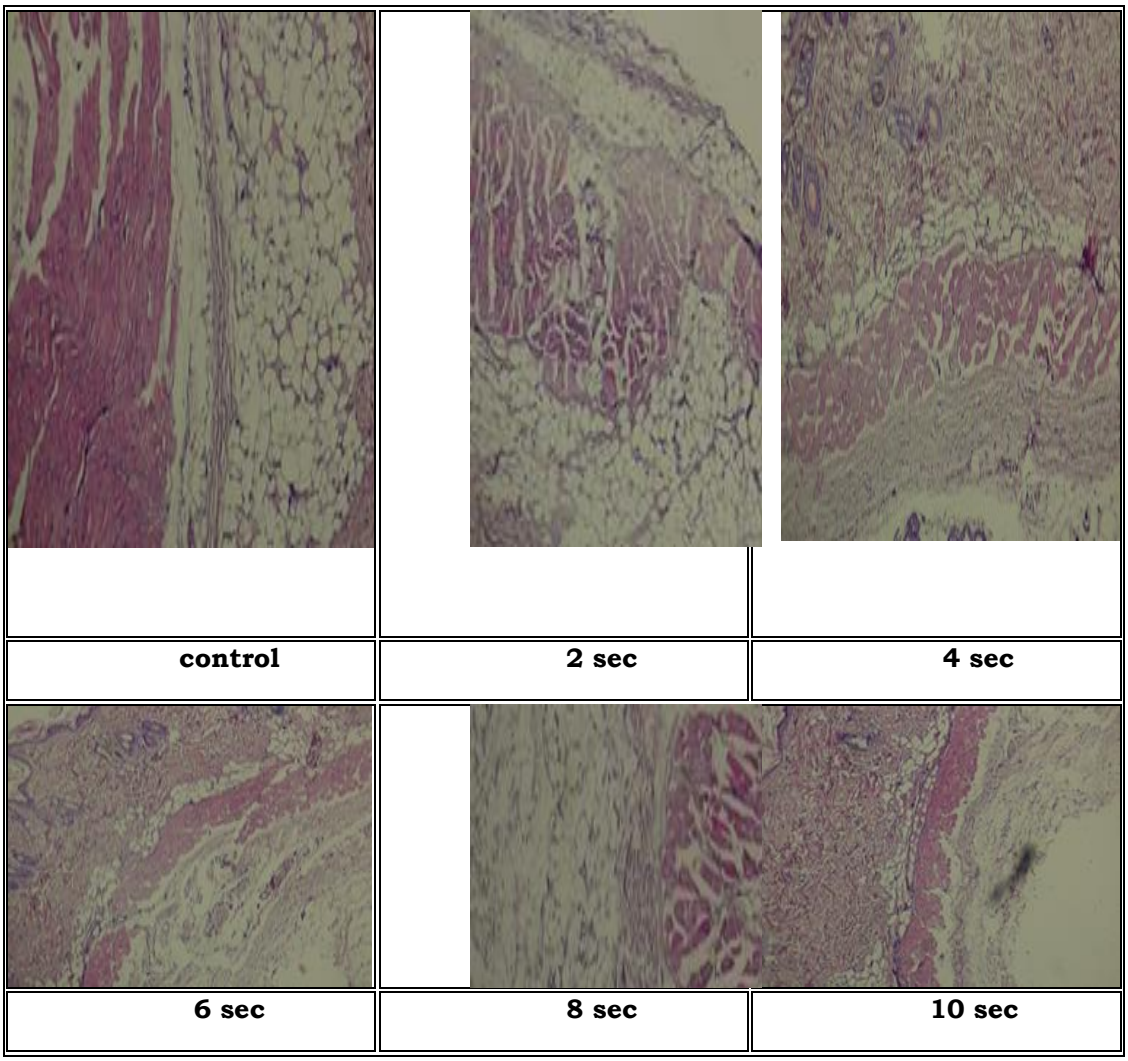

Figure (6) Histology images of skin rats sample after plasma exposure.

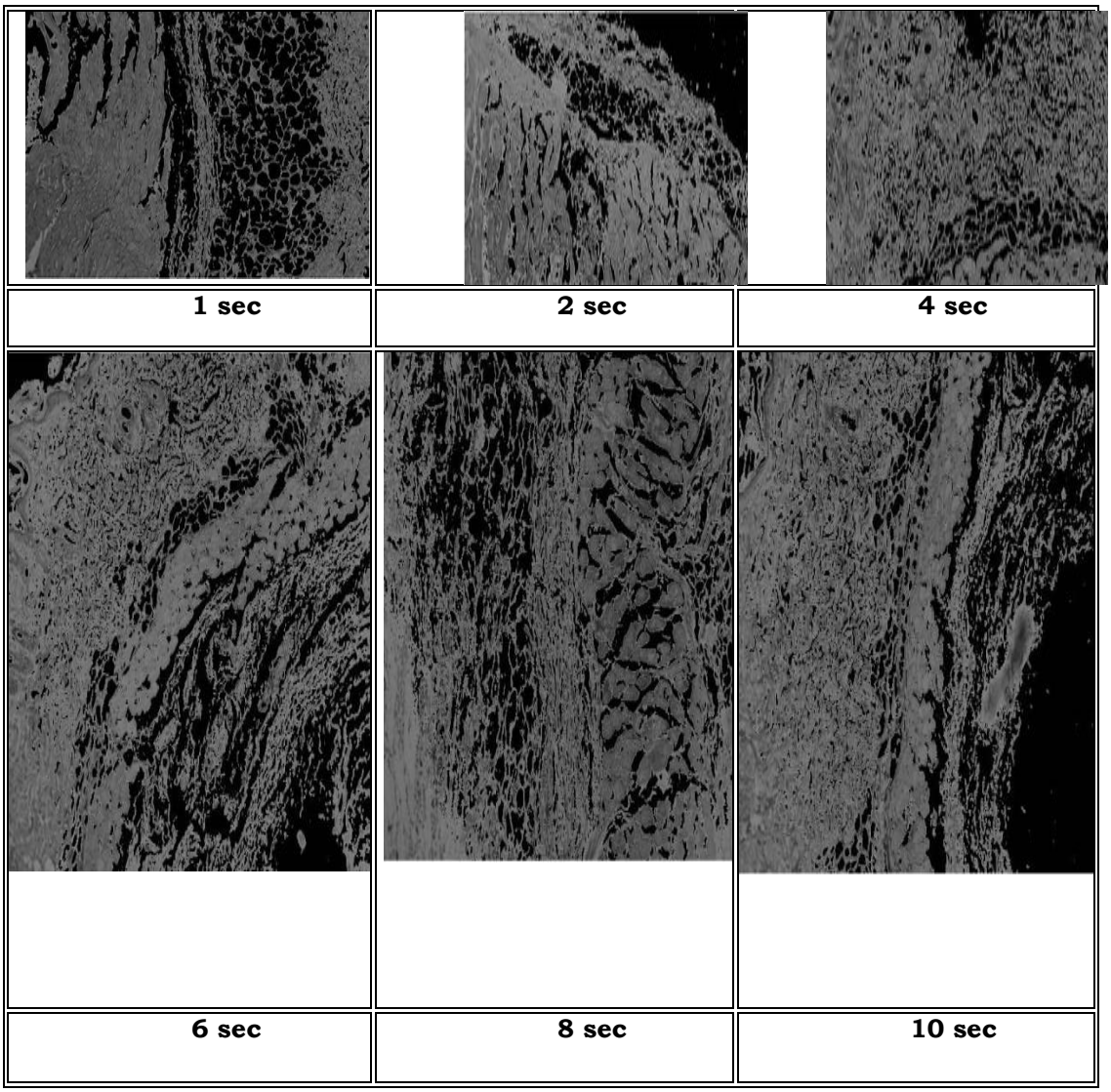

Figure (7) Threshold of Histology images of skin rats sample after plasma exposure.

Conclusions

The importance of the following work is the results obtained which can be useful for more applications in skin improvements. The results obtained in this works includes vitamin D levels in serum blood of rats, calcium levels in serum bloods, Triglycerides which is a type of fats, also studied and calculated in the same samples. Results calculated where supported by another comparison with histological images for skin samples. From results one can concludes that the cold plasma has a significant effect on skin texture and smoothing. Cold plasma increasing vitamin D levels and it is also affected on calcium levels after certain time of exposure and effects on skin fats by the same way.

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